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Original Article

FORMULATION AND EVALUATION OF A TOPICAL GEL CONTAINING MINOXIDIL AND TOFACITINIB CITRATE FOR ALOPECIA AREATA

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ABSTRACT

Objective: The objective of the present study is to formulate and evaluate a topical gel containing Minoxidil and Tofacitinib citrate for alopecia areata.

Methods: Six gels were formulated using the direct-dispersion method by using polymers in the ratio of Carbopol 934: HPMC and Carbopol 934: HPC in three different concentrations each. All the prepared gels were then characterized for its drug content, pH, Rheological measurement, Spreadibility, skin adhesion study, *In vitro* drug release, Ex-vivo skin permeation study and stability studies.

Results: All the six formulations were evaluated for various parameters such as pH, viscosity, spreadibility, and drug content and *in vitro* drug release. The pH of all the formulations was in the range of 6.3-6.8 which was optimum for the skin. As the concentration of the polymer increased the viscosity also increased, F6 had the highest viscosity among all of 1082 cps. F5 had the highest spreadibility of 4.3±0.15 cm and the drug content of all ranged between 88-96% of Minoxidil and 86-97% for Tofacitinib citrate with F5 giving the best result drug release across a cellulose membrane for a period 8 h of 94.22±0.19% for Minoxidil and 93.62±0.49% for Tofacitinib citrate. Formulation F2 and F5 were subjected to skin adhesion studies by the use of wistar rat skin with F5 giving the highest bioadhesion of 108 g/cm2. Formulation F5 was selected as an optimized formulation among the six as it gave the best results for all the parameters. Then the optimized formulation was subjected to an ex-vivo permeation study for a period of 8 h and by using wistar rat skin as a permeation barrier and the drug release for Minoxidil and Tofacitinib citrate was found to be 71.94±0.78% and 69.49±0.47%. The stability study was carried out for two months at accelerated condition i.e., 40 °C±2 °C/75±5% RH proved that the formulated gel was Stable.

Conclusion: The formulated topical gel containing Minoxidil and Tofacitinib citrate were found to be a promising approach for the treatment of alopecia areata.

Keywords: Minoxidil, Tofacitinib citrate, Alopecia areata, Topical gel

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INTRODUCTION

Alopecia areata (AA) is a non-scarring, recurrent, auto-immune and inflammatory scalp and/or body hair loss condition. AA is further characterized into two types; the first one is alopecia totalis which is hair loss of either patches or total hair loss of scalp and second one is alopecia universalis which is 100% hair loss of scalp and body hair. Hair loss can be caused by various factors such as genetics, hormones, stress, and infectious disease and so on [1]. But T-Lymphocytes play a key role in AA as they attack the hair follicles around them and cause inflammation and ultimately leading to hair loss [2].

Minoxidil (MNX) is used orally for the treatment of hypertension and was introduced in 1970s, but its most common side effect was hypertrichosis, including regrowth of hair in male balding. MNX a piperidino-pyrimidine derivative, is a potent arteriolar vasodilator as it is a potassium pump opener which is localised on the smooth muscular cells of the peripheral artery. Presently MNX is widely used for the treatment AA due to its most occurring side effect of hirsutism. Minoxidil helps in hair growth by either shortening the telogen phase or prolonging the anagen phase or a combination of both. When MNX is used topically, it prevents the shortening of hair in the anagen phase and prolonging this period, then regrowth of hair follicles by increasing the length and thickness of follicles in the anagen phase and lastly by reducing the telogen phase [3].

Tofacitinib citrate (TFC) is a Janus kinase (JAK) inhibitor. Recent studies have shown that JAK inhibitors are an upcoming treatment for AA due to their faster mechanism of action and lesser side effect. AA is caused when the hair follicles start presenting major histocompatibility complexes which take part in the activation of JAK-STAT pathway which leads to T-cell-mediated inflammation. JAK inhibitors block this pathway thus inhibiting the inflammation [4]. TFC was initially introduced in 2012 for the treatment of rheumatoid arthritis. TFC inhibits JAK3 enzyme therefore, it is used to treat various dermatological conditions which is regulated by JAK1/3, such as AA, psoriasis and dermatitis [5].

Various MNX formulations are available in the market, but when MNX is used alone it does not give a long-term effect therefore, it must be administered repeatedly, which leads to an adverse effect such as scalp dryness, irritation, burning sensation, redness and dermatitis [6, 7]. TFC, when used in combination of MNX will produce a long-term effect and will serve to overcome most of its adverse effects. In 2019, a study was conducted by using MNX in combination of TFC (oral conventional tablet formulation) proving that it leads to a synergistic activity [8].

In the present study, an attempt has been made to formulate and evaluate a gel containing MNX and TFC. Gels are semi-solid preparation consisting of a two-phase system in which the small organic molecules are merely dispersed throughout the continuous phase and large organic molecules are dissolved in the continuous phase [9]. The drug delivery to and via hair follicles is chosen as they are becoming very interesting target sites in the treatment of AA as they are surrounded by capillaries and antigen-presenting cells which are associated with the sebaceous glands so the direct action can be stimulated [10]. The gel was evaluated for the following: physical properties, pH, and viscosity, spreadability, *in vitro* and ex-vivo drug release, skin adhesion and stability.

MATERIALS AND METHODS

Materials

The active ingredient, MNX was obtained as a gift sample from Maruti Futuristic Pharma Pvt. Ltd., Bangalore, India and TFC was

obtained as a gift sample from Unichem Laboratories Ltd, Goa, India. The polymers Carbopol 934, HPMC K4m and HPC were procured from Yarrow Chem products, Mumbai. Dialysis membrane-150 was purchased from HI Media Laboratories Pvt. Ltd, Mumbai. All other chemicals were of the analytical grade.

Methods

Formulation of topical gel containing minoxidil and tofacitinib citrate: The compatibility study conducted by Differential scanning calorimeter and Infrared spectroscopy shows that there is no interaction between polymers and drugs. The polymers were employed in the ratio of Carbopol 934: HPMC K4m and Carbopol 934: HPC in three different concentrations each (table 1). Polymers were soaked separately overnight. Then the active ingredients, MNX was dissolved in Ethanol and TFC was dissolved in DMSO. Both of the solutions containing the drugs were then mixed together and propylene glycol and two drops of fragrance was added to it. Then the above solution was divided into 2 halves. The first half was added to the overnight-soaked carbopol polymer and the second half was added to the overnight-soaked HPMC or HPC polymer and stirred. The resultant solutions were later mixed together with continuous stirring using a propeller at 500 rpm for 10 min. Then TEA was added to the above gel in order to get the required consistency and to maintain the pH. Finally, preservatives were added and stirred for a minute. The composition of the topical gel containing MNX and TFC is reflected in table 1.

Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was performed and the DSC thermograms of pure drug MNX, pure drug TFC and their physical mixture with the polymers i.e. (MNX-TFC-carbopol 934-HPMC K4m) and (MNX-TFC-carbopol 934-HPC) are shown below in fig. 1, 2, 3 and 4 respectively. There were no substantial changes in the melting exotherm of MNX and melting endotherm of TFC in physical mixture which conclude that there are no interactions between the drugs and polymers there by the drugs and polymer was compatible with each other.

FTIR (Fourier Transform Infrared) spectroscopy

FTIR was performed and the FTIR spectrum of pure drug MNX, pure drug TFC, physical mixture of the pure drugs and their physical mixture with the polymers i.e. (MNX-TFC-carbopol 934-HPMC K4m) and (MNX-TFC-carbopol 934-HPC) are shown below in fig. 12, 13, 14, 15 and 16 respectively. Comparison was established between the obtained frequency and the standard functional group frequencies of MNX and TFC. The peaks obtained in the IR spectrum of a physical mixture of drugs and excipients had similar peaks as that of the standard. IR spectrums obtained were superimposing on each other, which concludes that there are no interactions between the drugs and polymers there by the drugs and polymer was compatible with each other. The frequencies corresponding to the functional groups are shown in table 5, 6, 7, 8 and 9.

Characterization of topical gel containing minoxidil and tofacitinib citrate

The formulated topical gels containing Minoxidil and Tofacitinib citrate were characterized for the following parameters–

Physical appearance

The gel formulations were evaluated for its colour, gelling capacity, homogeneity and grittiness.

Measurement of pH

The determination of the pH of the gel was carried out by using a digital pH meter. 1g of gel was dissolved in distilled water and stirred until a uniform dispersion and then the volume was made up to 100 ml with distilled water. The measurement of pH of the formulations was carried out in triplicates and the mean values were calculated [11].

Viscosity

The viscosity of the formulated gels was determined by using Brookfield digital DV-II+Pro viscometer, equipped with a T-bar spindle no. D4 at the temperature of 25±0.3 °C having the rotation speed of 50 rpm. The viscosity of the gels was measured in triplicate and the mean values were calculated [12].

Spreadibility study

The spreadibility of the formulated gels was determined by using modified Meera *et al.* (2010) [13] method by measuring the diameter of 1g of gel sandwiched between two petri plates. 1g of gel was accurately weighed and kept at the centre of the one petri plate and the second petri plate was carefully placed over it. A weight of 50g was kept on the plate and allowed the gel to spread for 2 min. The diameter of the gel was then measured with the help of measuring scale. The diameter was measured in triplicates and the mean values were calculated.

Drug content

The drug content of MNX and TFC in the formulation was determined by accurately weighing 0.5g of gel equivalent to 10 mg of MNX and TFC and dissolving in methanol in a 100 ml volumetric flask and sonicated for 2 h for proper dispersion of the gel. The solution was then filtered and absorbance was measured at 258 nm for MNX and 290 nm TFC using UV-VIS spectrophotometer and % drug content was calculated. Readings were taken in triplicates and mean values were calculated [14].

***In vitro* drug release**

In vitro drug release study of MNX and TFC through the formulated gels was performed by using Franz diffusion cells with 1.6 cm2 diffusion area and cellulose dialysis membrane-150 was used as a permeation barrier. The dialysis membrane was saturated in phosphate buffer saline (PBS) pH 7.4 for 24 h and then the membrane was clamped between the donor and the receptor compartment of Franz diffusion cell. 0.5g of gel equivalent to 10 mg of MNX and TFC was evenly applied on to the surface of cellulose dialysis membrane-150. Phosphate buffer saline (PBS) pH 7.4 was used as the dissolution medium and was filled in the receptor compartment; stirring of the solution was carried out using magnetic bead and the entire assembly was maintained at 37 °C±0.5 under constant magnetic stirring. The receptor chamber was covered with aluminium foil to prevent drying out. 3 ml of samples were withdrawn at predetermined time intervals over a time period of 8 h (0, 1, 2, 3, 4, 5, 6, 7 and 8 h) by replacing it with 3 ml of fresh PBS pH 7.4 to maintain the sink condition. Dilutions were made and the sample was analysed by UV-VIS spectrophotometer at 258 nm for MNX and 290 nm for TFC and %CDR was calculated. The readings were taken in triplicates and mean values of %CDR were used for plotting graphs [11, 15].

Skin adhesion test

The bioadhesive strength of the formulated gels was measured with the use modified Patel *et al.* [16] of wistar rat skin. The study protocol was approved by institutional animal ethical committee KLECOP Belagavi. The studies were conducted as per CPCSEA Guidelines. The animals were housed individually for atleast 7 d before an experiment to allow acratches, bites and small imperfections to heal. The hair of the rats was removed 3 d before from the date of commencement of the experiment. After sacrificing the animals by cervical dislocation, the animal skin were obtained. First subdermal fat and fascia were removed from the rat skin and the obtained skin was cleansed properly with a mild skin cleanser. The study was conducted by using a two-arm balance in which the left arm was tied with one glass slide with the cleansed rat skin placed on it with the area of 2 cm^2. Then 1g of formulated gel was applied on to the skin and then the second slide was placed on top of it by applying small pressure to remove air bubbles and kept for 5 min. 50 mg/min weight was added to the right pan slowly till the glass slide detached from the skin surface. Bioadhesive strength was calculated using the following formula. The measurement and skin adhesion studies were done in triplicates and mean values were calculated.

$$\text{Bioadhesive strength} = \text{weight required(g)/area(cm2)}$$

Ex-vivo skin permeation study

Ex-vivo drug release study of MNX and TFC through the formulated gel was performed by using Franz diffusion cells with 1.6 cm2 diffusion area. The permeation barrier used was wistar rat skin from the dorsal region. The hair, sub dermal fat and fascia were removed

from the rat skin and the obtained skin was cleansed properly with a mild skin cleanser. This skin was clamped between the donor and the receptor compartment of Franz diffusion cell with the stratum corneum facing upwards. 0.5g of gel equivalent to 10 mg of MNX and TFC was evenly applied on to the surface of skin. PBS pH 7.4 was used as the dissolution medium and was filled in the receptor compartment, stirring of the solution was carried out using magnetic bead and the entire assembly was maintained at 37 °C±0.5 under constant magnetic stirring. The receptor chamber was covered with aluminium foil to prevent drying out. 3 ml of samples were withdrawn at predetermined time intervals over a time period of 8 h (0, 1, 2, 3, 4, 5, 6, 7 and 8 h) by replacing it with 3 ml of fresh PBS pH 7.4 to maintain the sink condition. Dilutions were made and the sample was analysed by UV-VIS spectrophotometer at 258 nm for MNX and 290 nm for TFC and %CDR was calculated. The readings were taken in triplicates and mean values of %CDR were used for plotting graphs [11, 15].

Stability study

The optimized formulation F5 was used to perform stability studies. The stability study was carried out for two months at accelerated condition i.e. 40 °C±2 °C/75±5% RH. The evaluation parameters considered were physical appearance, pH, viscosity and drug content of Minoxidil and Tofacitinib citrate. The samples were withdrawn after every 15 d and evaluated for the above said parameters in triplicates [15, 17].

RESULTS AND DISCUSSION

Gel formulations were prepared with an intention of increasing the contact time of the drug with the scalp region so that minoxidil is released in a prolonged manner for an extended period of time.

Physical appearance

Prepared formulations were white to translucent in colour, having a smooth texture with no grittiness. From the results, it can be concluded that all the formulations showed good homogeneity and no grittiness. The formulations F1 TO F3 were translucent due to lack of HPC polymer. However the formulations F4 to F6 were transparent clear due to the presence of HPC. The results in the form of mean±SD are shown in table 2.

Measurement of pH

In order to prevent skin irritation, the pH of the formulated gel must be close to the skin pH as topical gels are directly applied on the skin. The pH of formulations was found to be in the range of 6.3-6.8, which correlates with the skin pH thus preventing skin irritation. F5 showed pH 6.75±0.04, which is nearer to the neutral pH and suitable for topical formulations. The results in the form of mean±SD are shown in table 3.

Viscosity

The viscosity of the formulated gels was determined using a Brookfield viscometer at of 25±0.3 °C. Viscosity of the gels was in the range of 409-1083 cps. It was observed that as the ratio and concentration of polymers increases the viscosity also increase. The viscosity values of the gels were increased in the following order. Carbopol 934: HPC>Carbopol 934: HPMC K4M [7]. The results in the form of mean±SD are shown in the table 3.

Spreadability study

The spreadibility of the formulated gels was found to be in the range of 3.5 to 4.3 cm and were found to be satisfactory and within the limit. The results are shown in the form of mean±SD in table 3.

Drug content

The percent drug content of formulated gels was found in the range of 88-96% for MNX and 87-97% for TFC. The results are in the form of mean±SD shown in table 3.

***In vitro* drug release**

The *in vitro* drug release of MNX and TFC from the formulated topical gels was performed using Franz diffusion cell for 8 h. When the drug release as compared by taking polymer into consideration the Carbopol 934: HPC ratio gave a higher drug release as compared to Carbopol 934: HPMC K4m. This may be explained by the fact that the viscosity of the gel matrix highly influences the drug release mechanism [18]. The variations in the drug release profiles from various gels can be explained based on the composition, viscosity and swelling ability of the polymers. The drug release was found to be high with Carbopol 934 and HPC polymers than Carbopol 934: HPMC K4m. Carbopol and HPMC possess more swelling ability than HPC. So HPC alone might have shown more drug release compared to Carbopol and HPMC [7]. After 8 h maximum release was shown by formulation F5 i.e., 94.22%±0.19% for MNX and 93.62%±0.49% for TFC as compared to other formulated gels. A graph of %CDR v/s time demonstrating the comparison was plotted for all the formulations in the form of mean±SD (fig. 14 and 15).

Skin adhesion test

The bioadhesive strength of the formulated gels was measured by two arm balance with the use of wistar rat skin of 2 cm^2 area. Bioadhesive strength is based on the composition, viscosity and mucoadhesive capacity of the polymers used to formulate gels. The skin adhesion study was performed on F2 and F5 formulations as they gave the best results among the six gel formulations. Bioadhesive strength was estimated using the following formula.

$$\text{Bioadhesive strength} = \text{weight required(g)/area(cm2)}$$

The bioadhesive strength of F2 and F5 is given in table 4 and the results were found to be satisfactory.

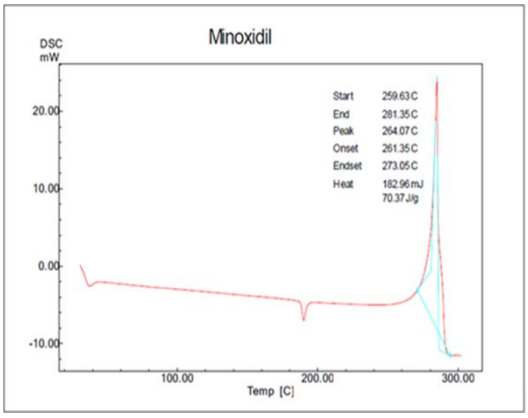

Fig. 1: Thermogram of Minoxidil pure drug

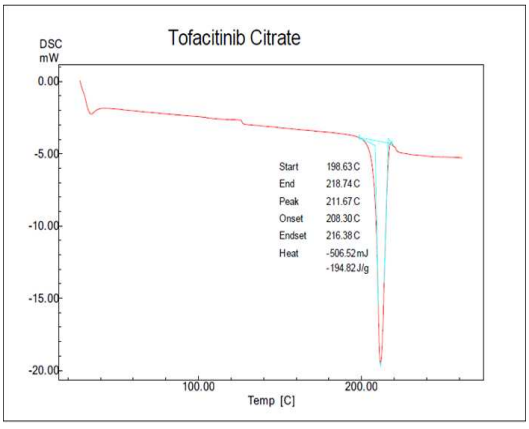

Fig. 2: Thermogram of Tofacitinib citrate pure drug

Ex-vivo skin permeation study

The ex-vivo permeation study was successfully carried out on the Franz diffusion cell for 8h using the optimized formulation F5

consisting of MNX and TFC. Both the drugs showed good drug release after 8 h and it was seen that as the time increased, the % cumulative drug release also increased. Addition of propylene glycol results in a supersaturated solution followed by precipitation of drug, leading to abrupt absorption patterns. Drug is absorbed from the site of application as long as it remains in solution form; for the same reason, gel formulations were prepared to get good permeability [7]. Graphs of %CDR v/s time demonstrating the drug release of Minoxidil (fig. 16) and Tofacitinib citrate (fig. 17) in the form of mean±SD are given below.

Stability study

The optimized formulation F5 was used to perform stability studies. The stability study was carried out for three months at accelerated conditions i.e., 40 oC±2 oC/75±5% RH. The evaluation parameters considered were physical appearance, pH, viscosity and drug content of MNX and TFC. All the results when the samples were withdrawn at an interval of 15 d for two months were found to be within limits with no significant variations ensuring stability of the formulations. The stability data is illustrated in the form of mean±SD in table 5.

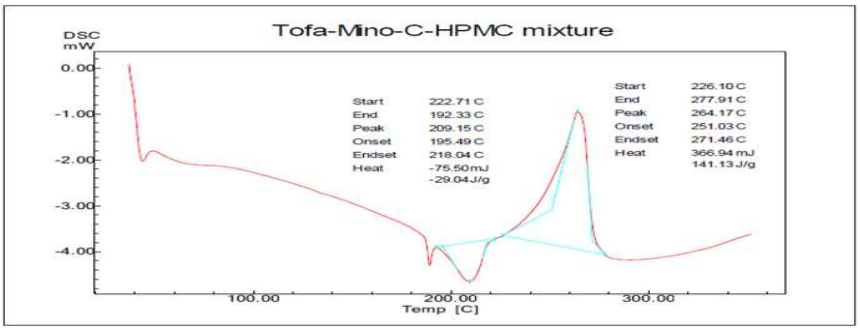

Fig. 3: Thermogram of physical mixture of Minoxidil+Tofacitinib citrate+Carbopol 934+HPMC K4m

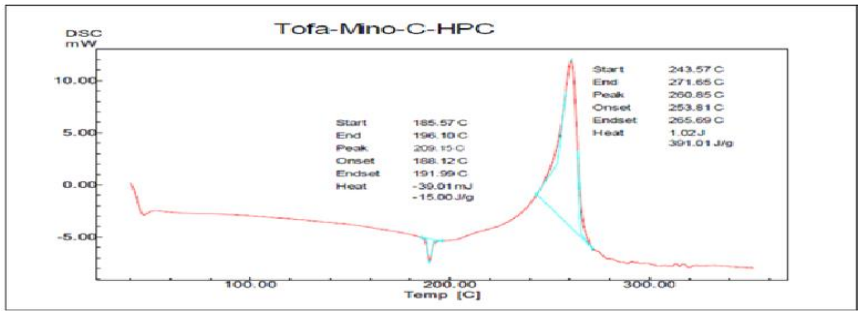

Fig. 4: Thermogram of physical mixture of Minoxidil+Tofacitinib citrate+Carbopol 934+HPC

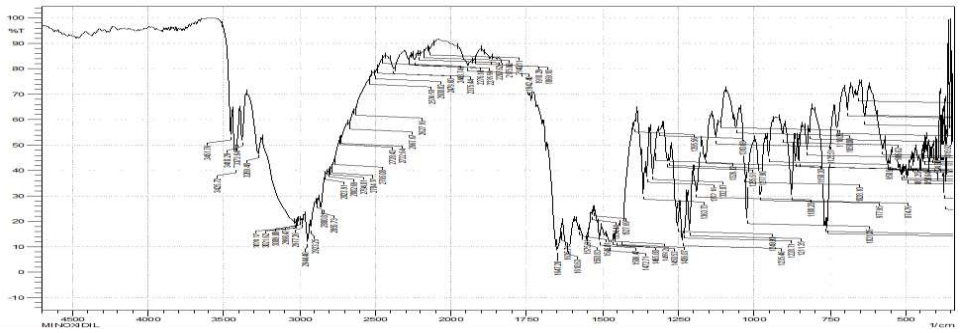

Fig. 5: FTIR spectrum of minoxidil

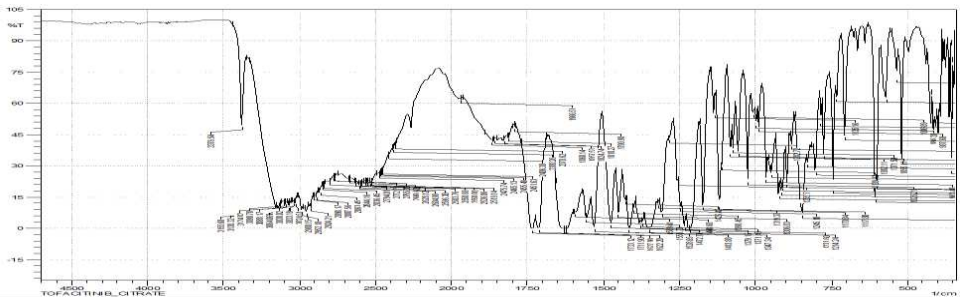

Fig. 6: FTIR spectrum of tofacitinib citrate

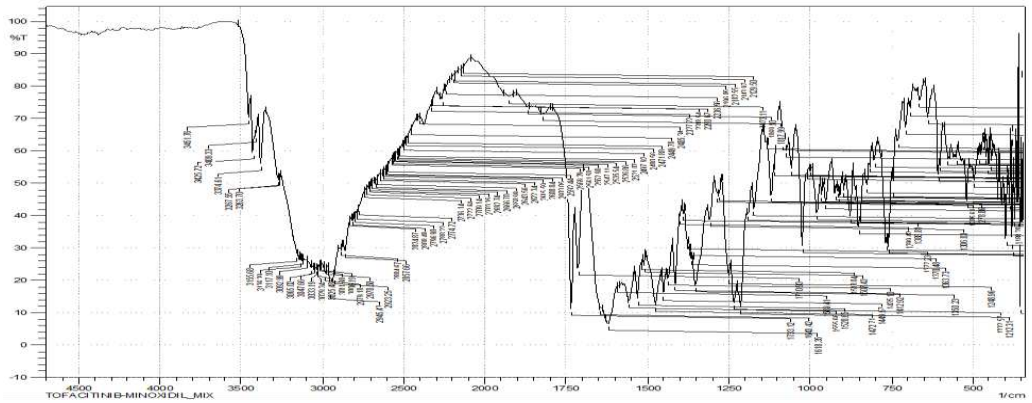

Fig. 7: FTIR spectrum of physical mixture of Minoxidil+Tofacitinib citrate

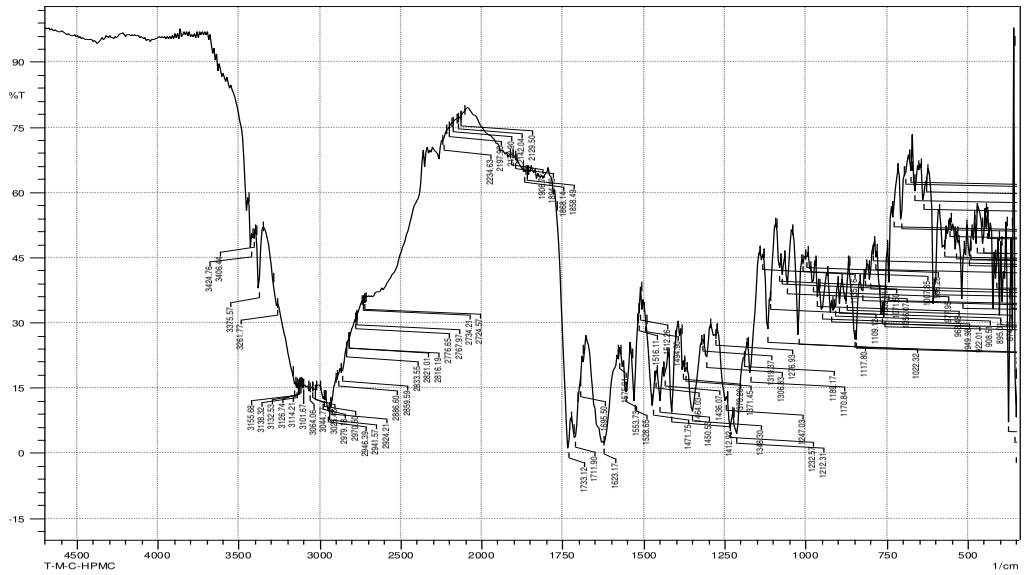

Fig. 8: FTIR spectrum of physical mixture of Minoxidil+Tofacitinib citrate+Carbopol 934+HPMC K4m

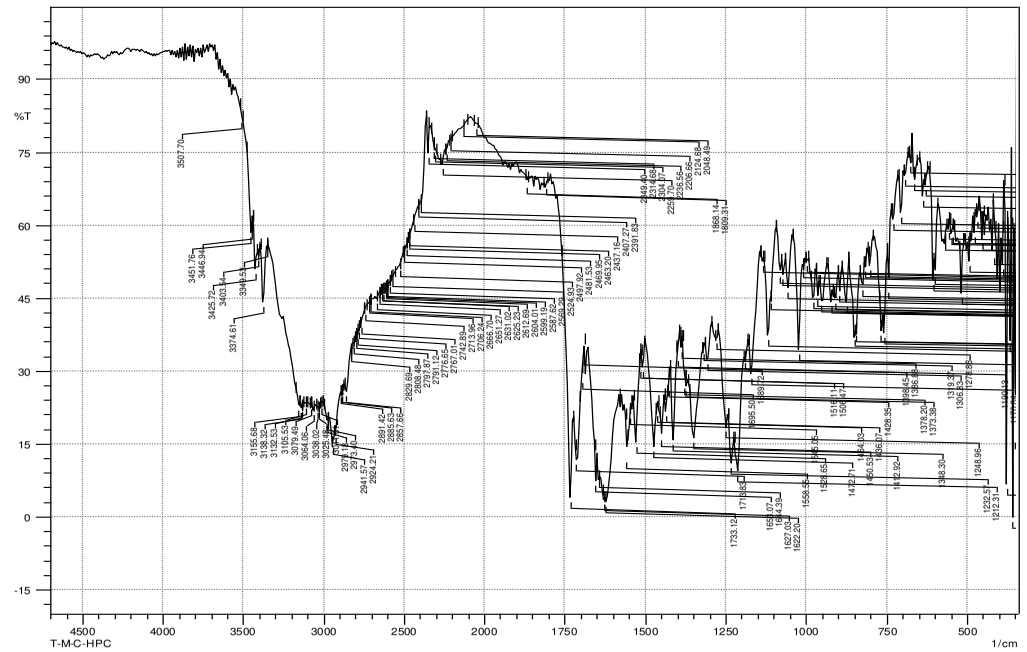

Fig. 9: FTIR spectrum of physical mixture of Minoxidil+Tofacitinib citrate+Carbopol 934+HP

Table 1: Formulation table of topical gel containing Minoxidil and Tofacitinib citrate

Ingredients	Formulations					
	F1	F2	F3	F4	F5	F6
Minoxidil (%)	2	2	2	2	2	2
Tofacitinib citrate (%)	2	2	2	2	2	2
Carbopol 934 (%)	1	1.25	1.5	1.25	1.5	1.75
HPMC K4m (%)	1	1.25	1.5	-	-	-
HPC (%)	-	-	-	1.25	1.5	1.75
DMSO (%)	20	20	20	20	20	20
Ethanol (%)	40	40	40	40	40	40
Propylene glycol (%)	10	10	10	10	10	10
Triethylamine	q. s	q. s	q. s	q. s	q. s	q. s
Methyl paraben (%)	0.1	0.1	0.1	0.1	0.1	0.1
Propyl paraben (%)	0.01	0.01	0.01	0.01	0.01	0.01
Fragrance (%)	0.1	0.1	0.1	0.1	0.1	0.1
Water	q. s	q. s	q. s	q. s	q. s	q. s
Total quantity (%)	100	100	100	100	100	100

Table 2: Evaluation of gels: physical appearance, homogeneity and grittiness

Formulation code	Physical appearance	Homogeneity	Grittiness
F1	White	+	-
F2	White	+++	-
F3	Translucent	+++	-
F4	Translucent	++	-
F5	Translucent	+++	-
F6	Translucent	+++	-

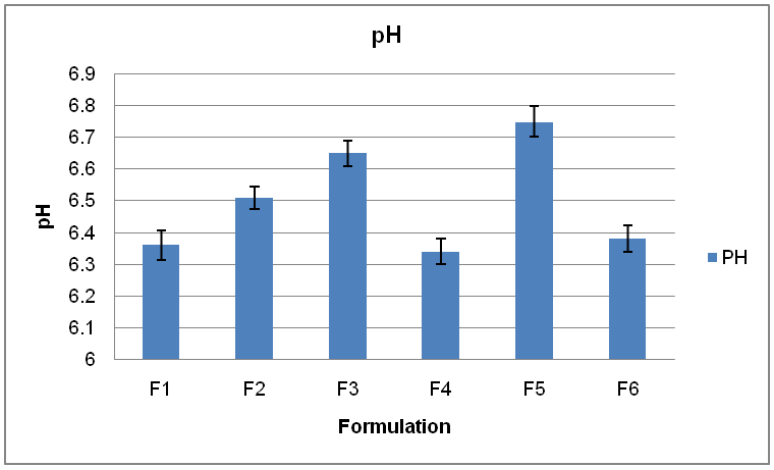

Fig. 10: Graph for pH of gel formulations (F1-F6) (n=3) (mean±SD)

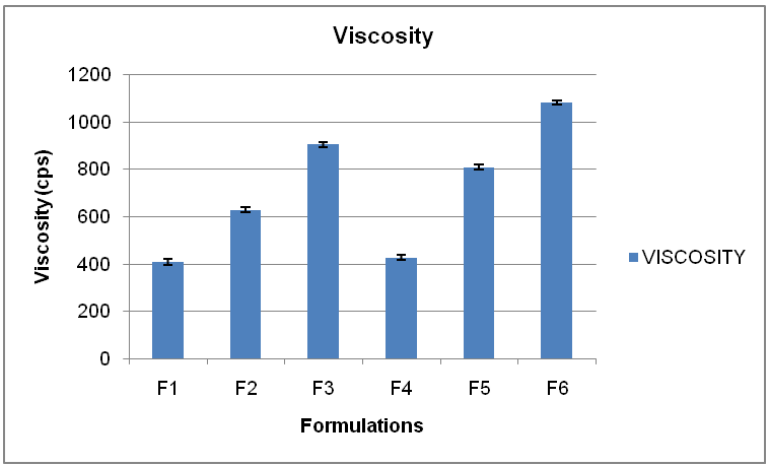

Fig. 11: Graph for viscosity (cps) of gel formulations (F1-F6) (n=3) (mean±SD)

Table 3: Evaluation of gels: pH, viscosity, spreadability and drug content (n=3) (mean±SD)

Formulation code	Ph	Viscosity	Spreadability	Drug content	
				Minoxidil	Tofacitinib citrate
F1	6.36±0.04	409.66±5.03	3.53±0.15	92.33±0.13	93.29±0.20
F2	6.51±0.03	629.33±4.04	3.5±0.26	94.55±0.34	95.38±0.32
F3	6.65±0.04	905.67±3.51	3.56±0.30	88.36±0.29	86.56±0.31
F4	6.34±0.04	427.33±3.51	3.76±0.15	88.50±0.21	89.42±0.33
F5	6.75±0.04	809.33±2.51	4.3±0.15	95.63±0.13	96.6±0.18
F6	6.38±0.04	1082±2.64	3.7±0.15	89.21±0.64	87.67±0.23

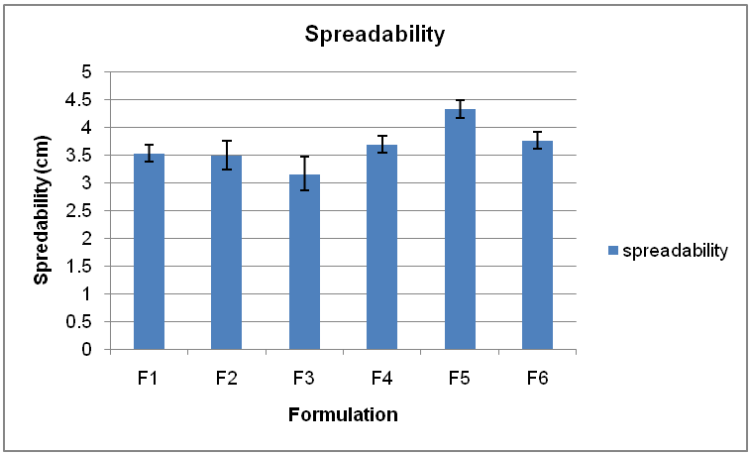

Fig. 12: Graph for spreadability (cm) of gel formulations (F1-F6) (n=3) (mean±SD)

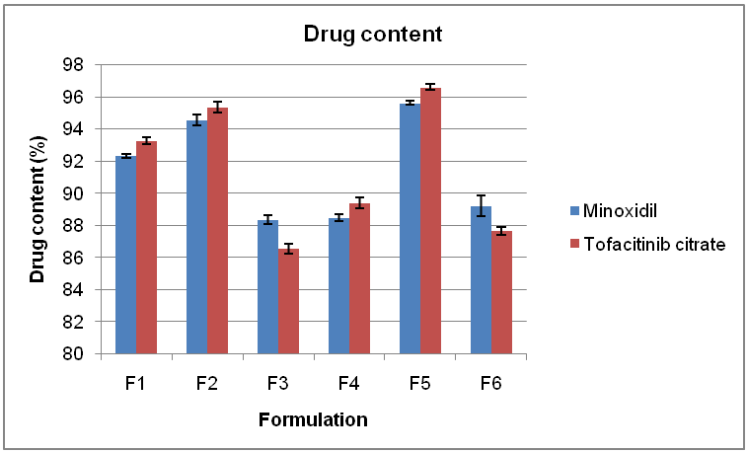

Fig. 13: Graph for drug content (%) of gel formulations (F1-F6) (n=3) (mean±SD)

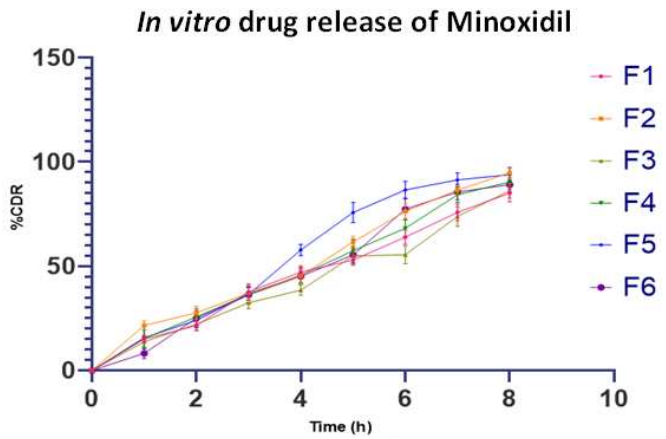

Fig. 14: *In vitro* drug release of Minoxidil from formulations F1-F6 (n=3) (mean±SD)

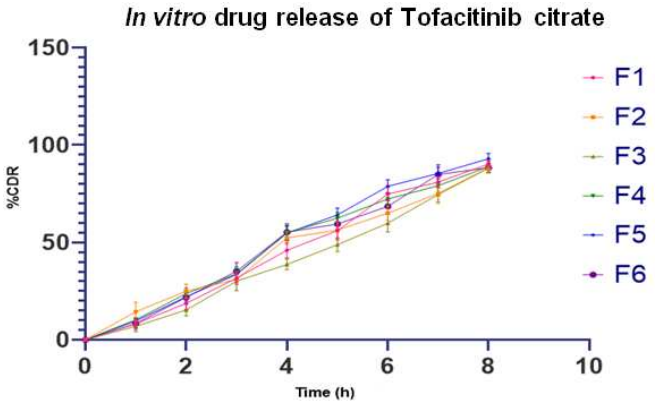

Fig. 15: *In vitro* drug release of Tofacitinib citrate from formulations F1-F6 (n=3) (mean±SD)

Table 4: Skin adhesion study (n=3) (mean±SD)

S. No.	Formulation code	Bioadhesive strength (g/cm²)
1	F2	103±2.08
2	F5	108±3.51

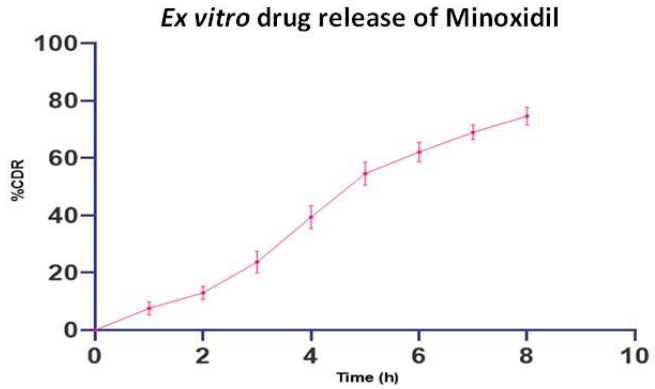

Fig. 16: *Ex-vivo* permeation study: % Cumulative drug release of Minoxidil from F5 (n=3) (mean±SD)

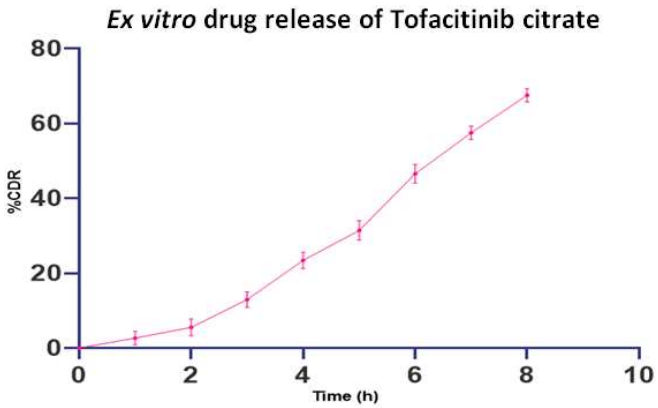

Fig. 17: *Ex-vivo* permeation study: % Cumulative drug release of Tofacitinib citrate from F5 (n=3) (mean±SD)

Table 5: Stability study data of F5 (n=3) (mean±SD)

	Physical appearance	Viscosity	pH	Drug content	
				Minoxidil	Tofacitinib citrate
Initial	Translucent	810.33±4.04	6.74±0.05	95.51 %	96.48 %
30 d	Translucent	810.67±3.51	6.73±0.05	95.27 %	95.82 %
60 d	Translucent	804.33±2.08	6.69±0.01	94.84 %	95.17 %
75 d	Translucent	791±3.51	6.69±0.05	94.15%	94.53 %
90 d	Slightly yellow	787.33±4.04	6.67±0.02	92.13 %	92.827 %

CONCLUSION

MNX and TFC were successfully incorporated in combination into the topical gel formulation for the treatment of Alopecia areata. Direct dispersion method was suitably applied for the formulation of topical gel using polymers of synthetic and semi-synthetic origins as gelling agents. The prepared formulations were subjected to various evaluation parameters such as physical appearance, pH determination, viscosity, spreadability, skin adhesion studies, drug content and *in vitro* drug release. The results of F5 formulation were best among all others hence selected as an optimized formulation for conducting ex vivo permeation study. Thus, we can conclude that the topical gel formulation containing MNX and TFC for the treatment of Alopecia areata can be successfully formulated by the direct dispersion method giving the results for all the evaluation parameters within the acceptable range.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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